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A SIMPLE COAGULOMETER

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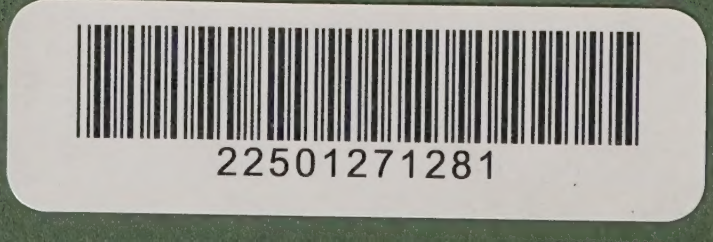
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A SIMPLE COAGULOMETER.[1]

By H. H. Dale and P. P. Laidlaw.

From the Wellcome Physiological Research Laboratories, Herne Hill, London, S.E.

We do not propose to give a review of methods previously suggested for estimating the coagulation-time of blood, as critical reviews of all the most important have been given in recent years by Addis (1908 [1]) and Morawitz (1911 [5]). Addis himself described a method with which he obtained results largely similar to those obtained with the method described below. His method, however, was far too elaborate and cumbersome to be used except in a laboratory, or a room specially set apart for such work. Our object is to describe a simple method, which could be used anywhere, involving no transport of large apparatus, but giving results apparently not inferior in self-consistency, and presumably, therefore, not inferior in accuracy to those obtained with more elaborate apparatus. We found the method inapplicable to the purpose for which we designed it, through circumstances having no connection with the method itself, but describe it in the hope that it may prove useful in studying the alteration of the coagulability of the blood in pathological conditions. It fulfils a number of conditions which appear to us important, and which are not all fulfilled by any other one method with which we are acquainted. These are the following. The observation is made on a single drop of blood; the area of contact with foreign bodies is practically constant in relation to the volume of blood examined, which is automatically measured; the blood is sealed from contact with air soon after shedding; the temperature is regulated by immersion in a water-bath; the observation is continuous, and the end-point is sharp and objective; the essential part of the apparatus is so simple and cheap that a new one is used for each observation. It has been one object of some methods to reduce contact with foreign bodies to a minimum, and it may be maintained that this is excessive in the case of ours. It seems to us a matter of little importance how much contact is involved, provided only that it be practically constant. The coagulation-time determined by any method has a value only relative to other determinations by the same method: it merely indicates the rate at which, under con-

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ditions which the method imposes, coagulation proceeds to an arbitrarily chosen end-point. The points of importance are, that the conditions should be constant and the end-point definite. It is claimed for some methods that one observer can, by practice, obtain results consistent among themselves. We shall consider our method worthless unless others, with a minimum of practice, and observing the conditions described, can obtain results consistent with ours. Our object being merely to suggest a method, we give only a few results so as to show the limits of its accuracy, we indicate the conditions which need careful observance, and illustrate the application of the method in examining the effect of certain substances reputed to affect the coagulation-time.

Description of the Method.

The essential part of our apparatus is a short length of capillary glass tubing. We found 2 cms. a convenient length, but this is purely a matter of convenience. The internal diameter is of more importance. We have been accustomed to draw out a supply of capillary tubing at the blow-pipe, cut into lengths of about 2 cms., and pick out those with internal diameters of 1·3 to 1·4 mm. For this sorting we used a gently tapering gauge, made from a steel crochet-hook, on which the two limiting diameters were found with a screw-gauge and marked with annular scratches. Great accuracy is not essential or practicable, but we do not doubt that the uniformity of results

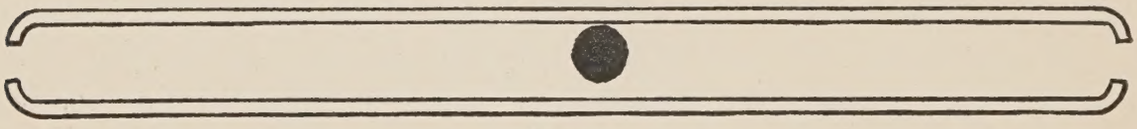

Fig. 1.

improves up to a point with the rigour of this selection. In each capillary is a clean leaden shot (see Fig. 1). Before this is introduced the capillary is narrowed in the flame at one end, just enough to prevent the passage of the shot. The shot being introduced at the other end, this is similarly narrowed, so that the shot can roll the whole length of the tube, but cannot fall out at either end. The size of the shot should be uniform, again, within certain limits, and must be so chosen that it will move quite freely up and down the tube, and yet be clearly visible when the latter is filled with blood. We find that a shot weighing 9 mgrms. is of suitable size for a tube of the diameter quoted above. Sufficient accuracy can be obtained by choosing from a packet of mixed small shot one of this weight, and picking out others which are not visibly larger or smaller than this standard. If the apparatus were to be used for a determination in which all the factors were strictly constant, it would doubtless be desirable to calibrate the tubes accurately and to choose shot of exactly uniform weight. In determining the coagulation-time of a drop of blood obtained by a finger-prick we believe that greater accuracy than that indicated would entail wasted labour. The shot should be clean, and misshapen ones should not be used. If necessary they can be cleaned by washing with dilute nitric acid, water, alcohol, and ether, and then drying. They should then be kept in a clean dust-tight box till required.

This capillary tube with its contained shot is the essential part of the apparatus, and a new one is taken for each determination. A basin of water with a thermometer is required, and, provided that the temperature chosen is between 35° and 40° C., within which range the temperature coefficient is

small, the temperature can be kept quite sufficiently uniform by adding small quantities of hot water from a kettle as required and stirring thoroughly. A good light is essential and a white basin should be chosen. If this be placed near a bright window light the reading can be made without difficulty. We have obtained excellent illumination by allowing an electric glow-lamp to hang partly immersed in the water, and recommend this arrangement where it is available. This is the only essential apparatus; the tubes can be held in the fingers and the ends closed by the clean thumb and finger of the observer for immersion in the constant-temperature bath. It is convenient, however, to have a small pair of tongs of some kind for holding the tube while filling, and a spring clip with the jaws furnished with little cups for holding clean plasticine, the filled tube being placed longitudinally between the opened jaws, so that when these approximate the tube is held with its ends sealed with plasticine (Fig. 2). The clip with the tube is then plunged

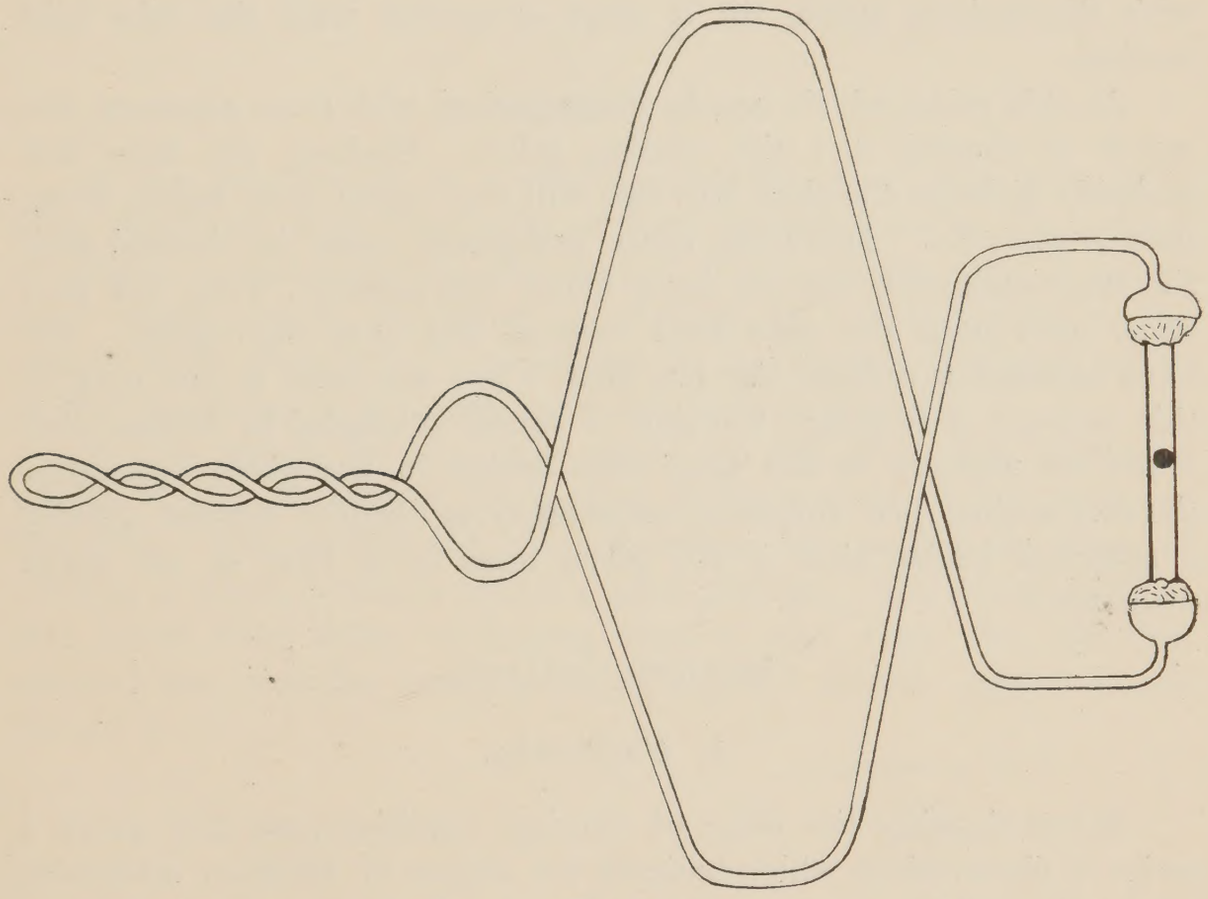

FIG. 2.

into the bath and the handle of the clip serves for further manipulation. The blood is obtained in the usual way by pricking a carefully cleaned finger in which passive congestion has been produced by swinging the arm and binding. The prick should be made with a perfectly sharp clean needle.

In our experiments the subject has pricked his own finger, the observer starting a stop-watch as soon as the spot of blood appears. A capillary is held ready, either in the fingers or with the small pair of tongs, and one end is brought into contact with the drop of blood, the tube being held with a gentle upward inclination. As soon as it is filled it is placed in the clip, the jaws of which have been prepared with clean plasticine, and the clip with the tube is immersed in the bath. Not more than 10″ should elapse between appearance of the

blood and immersion of the tube in the bath. With a little practice 6″ to 8″ is enough. By turning the handle of the clip between the fingers, the tube is rotated to and fro so that the shot runs gently up and down. If the relations of size between shot and tube given above are adhered to, the shot runs easily and is clearly visible in a good light. For about four-fifths of the time between the shedding and coagulation of the blood, the tube can be held nearly horizontal in the bath, the running of the shot being secured by slight tilting to and fro. Then an almost sudden increase in viscosity of the blood is observed, and presently the tube has to be held almost vertical to keep the shot travelling. Unless the tube is jerked or knocked, however, the shot should continue to travel smoothly up and down, but with diminishing speed, till it stops altogether with the tube held vertical.

At this point, which can be distinguished with some accuracy, the watch is stopped and the reading taken. Shaking the tube will probably dislodge the shot, but this will stop again after falling along part of the tube. Doubtless other end-points could be devised with the same apparatus, but we have found this moment, when the shot stops dead with the tube held vertical, the most convenient. We have repeatedly verified the fact that, when the tube is cut open at this moment of stoppage, the shot is found entangled in delicate clot. What we measure is the time after which, in the given apparatus, the clot attains just sufficient consistency to support against gravity a spherical leaden shot of the given mass in a tube of the given diameter.

Results Obtained.

A. *Uniformity.*

Before showing the effect of varying conditions, we may quote a series of observations which indicate the degree of accuracy attainable when all conditions are constant. We usually took a group of from four to six readings, in as rapid succession as convenient, and took the mean as the figure for one observation. A number of such groups are quoted in succeeding sections, and the scale of accuracy can be gathered from an inspection of these groups. The readings so obtained from any one individual at a given temperature showed a remarkable consistency over a period of days, when taken at different times in the day and with varying relation to meal-times. Thus in one individual twenty-four readings, in five groups, were taken at 38° C. over a period of ten days. The highest individual reading was 1′ 50″, the lowest 1′ 32″, the average of the twenty-four readings 1′ 41·5″, the mean error 4·3″. The consistency appears more striking if we compare the mean values obtained by averaging each group of readings separately, the five values being 1′ 41″, 1′ 42″, 1′ 42″, 1′ 42″,

1′41″. It cannot be doubted, indeed, that a mere comparison of these means gives an exaggerated impression of accuracy, which must be attributed to the fact that the occurrence of an exceptionally low reading in any group was in this series by chance balanced by the occurrence of an exceptionally high one. It should be stated, however, that this adjustment was not due to unconscious bias on the part of the observer, since the stop-watch was held by an independent onlooker, so that each group of readings was hidden from the observer until complete. The observation that coagulation-time is constant for the normal individual and independent of the time of day, meal-times, etc., is in accordance with the findings of Addis (1908 [1]) and does not agree with those of Coleman (1907 [2]).

B. *Sources of Error.*

If the temperature is carefully regulated, error can arise only from variations in the capillaries or from imperfections in the method of obtaining and collecting the drop of blood. These two classes of error really reduce themselves in practice to (1) variations in the weight of the shot, and (2) variations in the amount of kinase added to the blood during shedding, collection, and observation.

1. The weight of the shot varies as the cube, the resistance offered by the viscosity of the medium as the square, of the radius of the shot. The smaller the shot, therefore, the earlier the stage of clotting at which it will be upheld against gravity. The following series was made with tubes containing smaller shot than the standard adopted, the variation being probably round 7 mgrms. instead of the normal 9.

Temperature, 38° C.

	Error.
1′35″	+3″
1′29″	−3″
1′31″	−1″
1′32″	−0″
1′34″	+2″
1′31″	−1″
1′34″	+2″
Mean, 1′32″	±2″

This very good set of readings, from the point of view of self-consistency, was obtained from the individual who as consistently, with the standard shot, gave a mean time of 1′42″ at 38° C.

2. Apart from the ordinary precautions as to cleaning the finger carefully, and using a new tube for each observation, keeping the prepared tubes free from dust, etc., we must emphasize the importance of having a perfectly sharp needle. The following series, made on an individual, whose normal reading at this temperature was 1′39″, shows that, with a needle, which, though clean, had the point turned,

the amount of kinase added from the tissue wounded by the puncture is not only liable to be excessive, but is irregular, so that the mean value is low, and the mean error large. A series made next morning with a clean needle is shown for comparison.

SUBJECT, L. W. C.—*Temperature*, 40°.

Needle with Hooked Point.		New Needle.	
	Error.		Error.
1′22″	− 9″	1′45″	+6″
1′34″	+ 3″	1′37″	−2″
1′20″	−11″	1′37″	−2″
1′35″	+ 4″	1′39″	0″
1′44″	+13″	1′39″	0″
		1′41″	+2″
Mean, 1′31″	± 8″	Mean, 1′39″	±2″

We always used a fresh finger for each reading in a series. If more than one series of readings was made in a day, an interval of some hours was allowed to elapse, and the fingers cleaned with special care. Traces of blood left from a previous puncture will of course vitiate the result completely. There is no need to aim at uniformity in size of the drops of blood: the capillary will take up the required amount automatically. It is desirable, however, that the puncture should be so made that the blood wells out freely: if the blood oozes out slowly, or needs expression, the readings will be too short.

C. *Effect of Temperature.*

If the coagulation-time be taken at a number of temperatures in the neighbourhood of the normal body temperature, it is found that there is little variation over a range from about 35° to 45° C. Below 35°, however, the curve on which coagulation-times are plotted, rises more and more steeply. Below about 22° the temperature coefficient becomes very large. Fig. 3 shows the curve obtained by plotting coagulation-times as ordinates and temperatures as abscissæ, the points marked thus ⊙ being all from one individual (L. W. C.). The points shown thus ◈ were obtained by observations on another individual (P. P. L.) at an earlier stage of the work, when we took less precaution in the selection of tubes and shot. Our results are on the whole similar to those of Addis, though they lie nearer to a smooth curve. The only point of marked difference is, that he finds that the coagulation-time begins to get larger again above about 40°. We find that it is practically the same at 45° as at 40°; but it is admitted that, in the method used by Addis, agglutination of the corpuscles above 40° C. makes the determination of the end-point inaccurate.

Above 45° we find that the coagulation-time, as measured by our method, rapidly becomes larger again. Thus in two individuals the blood clotted at 45° in 1′ 39″ and 1′ 44″ respectively. The former of these gave a mean time of 2′ 5″ at 49°, the latter also 2′ 5″ at 50°.

The following are samples of the groups of readings from which the curve in Fig. 3 is drawn :—

April 20, 1911.—SUBJECT, L. W. C.—*Temperature of Bath*, 40° C.

Sample.	Coagulation-Time.	Error.
1 . .	1′ 45″	+ 6″
2 . .	1′ 37″	− 2″
3 . .	1′ 37″	− 2″
4 . .	1′ 39″	0″
5 . .	1′ 39″	0″
6 . .	1′ 41″	+ 2″
	Mean, 1′ 39″	Mean error, ± 2″
		Per cent. mean error, 2

March 22, 1911.—SUBJECT, L. W. C.—*Temperature of Bath*, 38° C.

Sample.	Coagulation-Time.	Error.
1 . .	1′ 46″	+ 4″
2 . .	1′ 46″	+ 4″
3 . .	1′ 36″	− 6″
4 . .	1′ 42″	0″
5 . .	1′ 45″	+ 3″
6 . .	1′ 36″	− 6″
	Mean, 1′ 42″	Mean error, ± 4″
		Per cent. mean error, 4

March 30, 1911.—SUBJECT, L. W. C.—*Temperature of Bath*, 35° C.

Sample.	Coagulation-Time.	Error.
1 . .	1′ 44″	− 7″
2 . .	1′ 58″	+ 7″
3 . .	1′ 50″	+ 1″
4 . .	1′ 51″	0″
5 . .	1′ 53″	+ 2″
	Mean, 1′ 51″	Mean error, ± 3·4″
		Per cent. mean error, 3

At temperatures somewhat lower, when the slight variations of temperature cause larger variations in readings and the end-point is not so sharply defined, the errors of individual readings are larger, but in our experience the mean of any five or six consecutive observations is fairly constant.

April 10, 1911.—Subject, L. W. C.—*Temperature*, 22° C.

Sample.	Coagulation-Time.	Error.
1 . .	4′ 57″	−16″
2 . .	5′ 8″	− 5″
3 . .	5′ 25″	+13″
4 . .	5′ 22″	+ 9″
5 . .	5′ 11″	+ 2″
	Mean, 5′ 13″	Mean error, ± 9″
		Per cent. mean error, 2·8

February 24, 1911.—Subject, P. P. L.—*Temperature*, 19° C.

Sample.	Coagulation-Time.	Error.
1 . .	7′ 35″	+ 3″
2 . .	8′ 4″	+32″
3 . .	7′ 41″	+ 9″
4 . .	7′ 37″	− 5″
5 . .	7′ 12″	−20″
6 . .	7′ 7″	−25″
	Mean, 7′ 32″	Mean error, ±16″
		Per cent. mean error, 2·5

April 7, 1911.—Subject, L. W. C.—*Temperature*, 25° C.

Sample.	Coagulation-Time.	Error.
1 . .	4′ 4″	+12″
2 . .	3′ 47″	+ 5″
3 . .	3′ 46″	− 6″
4 . .	3′ 50″	− 2″
5 . .	3′ 53″	− 1″
	Mean, 3′ 52″	Mean error, ± 5″
		Per cent. mean error, 2

The smallness of the temperature coefficient between 37° and 40° C. seems to us a very clear indication in favour of adopting some

temperature within this range, as the standard for comparative observations. Addis adopted 18°, as he considered that, with coagulation-time the larger errors would be proportionally less significant. We find, however, that differences are magnified with increase of the coagulation-time, partly because the end-point loses its sharpness as the process

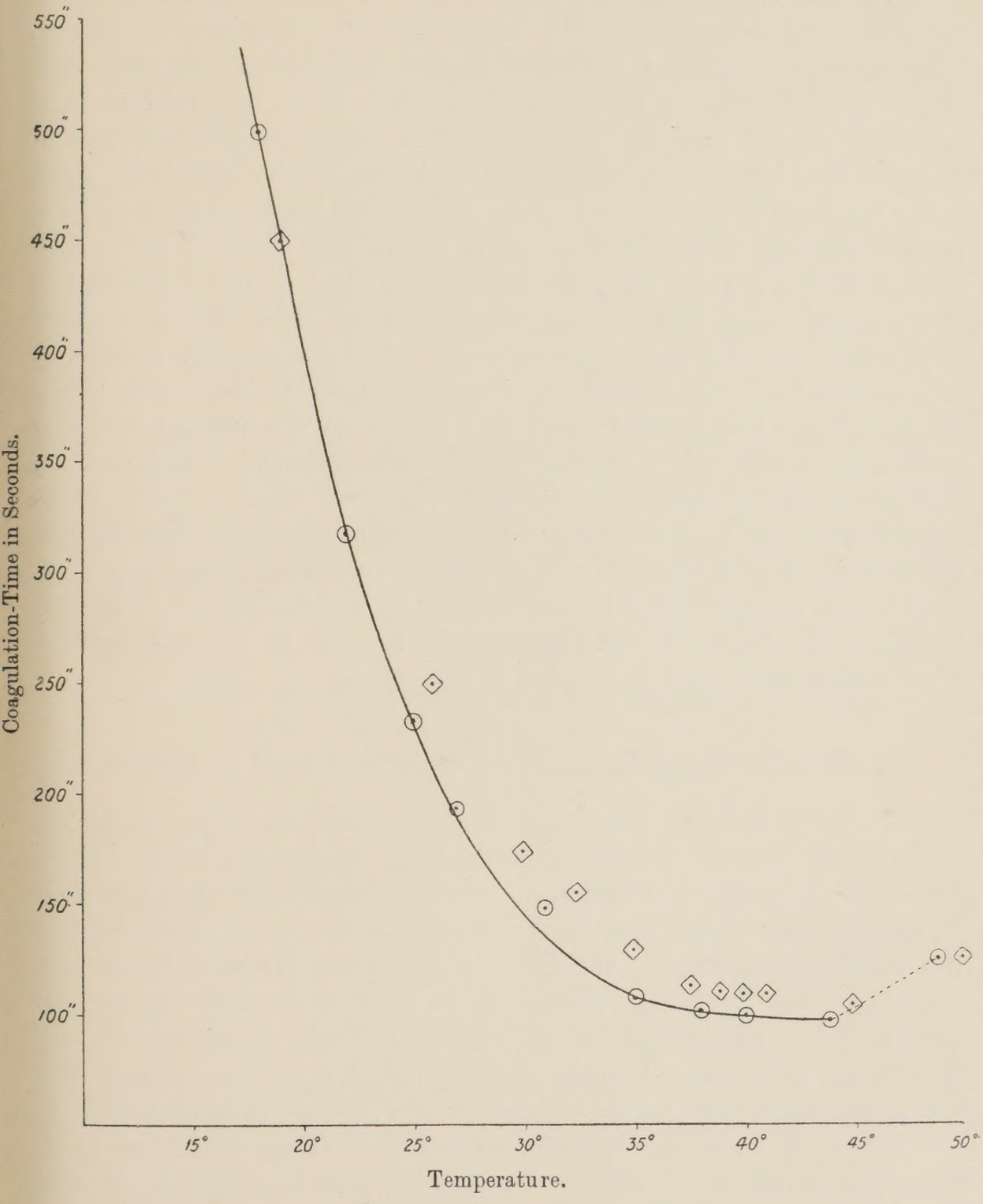

FIG. 3.

becomes slower. The effects of the small differences in pricking the finger and collecting the blood, which cause real differences in the clotting-times of successive specimens, will be magnified with the slowing of the rate of coagulation as well as the actual error in determining the end-point. The percentage variation from the mean may be expected,

therefore, to be about as great at the lower as at the higher temperature, and we find that it is so.

D. *Individual Variation.*

We have stated that, for a given normal individual, the coagulation-time, as measured by this method, shows surprising constancy. The variations between different normal individuals are also very small in the subjects we have examined. Thus one subject gave regularly at 38°, as we have shown, a mean coagulation-time of 1′41″ to 1′42″. Another individual, whenever examined, gave mean readings of from 1′45″ to 1′52″ at the same temperature, the most frequently occurring mean value in his case being 1′48″. The curves in Fig. 3 show that the coagulation-time of one individual is pretty consistently, though only slightly, larger than that of another over a range of different temperatures. At the same time it must be pointed out that these differences are well within the limit of accuracy attempted by most methods, and that it is only the average values of about six readings which show them with our method. The individual readings of the subject with the shorter average overlap those of the subject with the longer. It seems that about one and three-quarter minutes is, at any rate, a very common coagulation-time in normal individuals, as determined by our method at 37° to 38° C. We have examined the blood of eight persons in all, and in no case was the mean value more than $\pm$ 5″ wide of this value.

E. *Attempts to modify Coagulability in the normal Individual.*

We have already mentioned the fact that we were unable to detect any normal diurnal variations in coagulation-time. There remained the question, whether our method would show the alterations in coagulability which have been described as the result of administering certain substances. Wright and Paramore (1905 [3]) found that the coagulation-time was reduced to as little as one-fifth the normal period by the administration of calcium chloride or lactate by the mouth, or by adding 2 pints of milk per day to the normal diet. Other observers have failed to observe any change in coagulability as the result of such calcium-administration (1908 [1], 1907 [2]). It is clear that a very much smaller change than that described by Wright should be shown with our method if it occurred; we should not hesitate to regard as significant a change of 30″ in either direction in the average value, since this would be beyond the widest limit of error, even with the use of unstandardised shot and tubes. We have tried the effect of calcium in two cases, and of milk administration for a week in a third case, without detecting any change at all.

Experiment I.

In this experiment, two readings were taken just before calcium was administered, and groups of three readings two and three hours later.

March 16, 1911.—SUBJECT, H. H. D.—*Temperature*, 37° C.

Sample.	Time.	Coagulation-Time (one reading).		Variation from Mean during the Three Hours.
1 . .	12·50 a.m.	1′ 43″		− 5″
2 . .	12·55 ,,	1′ 43″		− 5″
At 1 o'olock, 30 grs. calcium lactate and little water, followed by light lunch.				
3 . .	2.50 ,,	1′ 49″	1′48″	+ 1″
4 . .	2.55 ,,	1′ 46″		− 2″
5 . .	3·0 ,,	1′ 49″		+ 1″
6 . .	3.55 ,,	1′ 52″	1′47″	+ 4″
7 . .	4.0 ,,	1′ 44″		− 4″
8 . .	4.5 ,,	1′ 46″		− 2″
		Mean, 1′ 48″		± 3″

On 17th March, at about mid-day, the average of four determinations was still 1′ 48″. It is evident that in this case calcium lactate had no effect on the coagulation-time.

Experiment II.

March 20, 1911.—SUBJECT, F. W. —*Temperature*, 38° C.

Sample.	Time.	Coagulation-Time.	Error.
1 . .	11.6 a.m.	1′ 46″	+ 1″
2 . .	11.10 ,,	1′ 45″	0″
3 . .	11.15 ,,	1′ 58″	+ 13″
4 . .	11.18 ,,	1′ 39″	− 6″
5 . .	11.22 ,,	1′ 46″	+ 1″
6 . .	11.25 ,,	1′ 35″	− 10″
		Mean, 1′45″	Mean error, ± 5″

At 11.45, 60 grns. calcium lactate by mouth.

At 2.20 p.m. to 2.45 p.m., six observations were made, and the mean coagulation-time was 1′ 48″ with a mean error of ± 5″.

At 5.50 p.m. to 6.15 p.m. six observations were made and the mean coagulation-time was 1′ 52″ with a mean error of 3′ 5″.

On 21st March, at mid-day, four observations were made, and the average coagulation-time was 1′ 48″ with a mean error of 4·5″.

In this case, again, calcium lactate produced no effect which could be regarded as outside the limits of error. The small apparent difference, if it were regarded as significant, would indicate delay rather than acceleration of clotting. The third subject (L. W. C.) took

2 pints of milk daily for nine days, and during this period the coagulation-time was estimated five times, each determination being the mean of four to six readings. His coagulation-time before taking milk was 1′ 41″. The determinations during the milk-period were—third day, 1′ 42″; fifth day, 1′ 42″; eighth day, 1′ 42″; ninth day, 1′ 41″. We do not suggest, of course, that these results prove that, in cases of slow coagulation, due to deficient calcium, the administration of calcium would produce no effect. They simply show that, in these normal individuals, our method shows no such change as the result of giving calcium as Wright observed with his.

Von den Velden (1911 [4]) has described a great and rapid increase of the rate of coagulation as the result of the administration of adrenine by the mouth, the coagulation-time being reduced to about one-half of the normal. Our one experiment made to test the point was completely negative.

Experiment III.

March, 7, 1911.—Subject, P. P. L.—*Temperature*, 40° C.

Sample.	Time.	Coagulation-Time.	Variation from the Mean.
1 . .	3.48 p.m.	1′ 39″	− 4″
2 . .	3.52 ,,	1′ 47″	+ 4″
3 . .	3.57 ,,	1′ 42″	− 1″
Tea.			
4 . .	4.50 ,,	1′ 48″	+ 5″
4.55 p.m., 0·5 mgrm. adrenine in 50 c.c. water.			
5 . .	5.2 p.m.	1′ 41″	− 2″ (Pulse, 80)
6 . .	5.25 ,,	1′ 42″	− 1″
7 . .	5.43 ,,	1′ 35″	− 8″
8 . .	5.47 ,,	1′ 36″	− 7″ (Pulse, 82)
9 . .	5.56 ,,	1′ 54″	+11″
10 . .	6.0 ,,	1′ 42″	− 1″
11 . .	6.12 ,,	1′ 50″	+ 7″
		Mean, 1′ 43″	

The average coagulation-time in this series was 1′ 43″, and the average variation from the mean was ± 5″. These figures are precisely similar to a normal series, and it is difficult to believe that adrenine had any effect on the rate of coagulation.

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